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Detection of Varicella-Zoster Virus DNA in Peripheral Mononuclear Cells From Patients With Ramsay Hunt Syndrome or Zoster Sine Herpete

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On the basis of alterations in varicella-zoster virus (VZV) antibody titers, it appears that Bell's palsy in some patients could be associated with VZV reactivation, that is, zoster sine herpete. To obtain stronger evidence of this association, polymerase chain reaction (PCR) was used to detect VZV DNA in auricular lesions or peripheral blood mononuclear cells (PBMCs) from Bell's palsy or Ramsay Hunt syndrome patients. VZV DNA was detected in the auricular lesions of Ramsay Hunt syndrome, in PBMCs from 2 Ramsay Hunt syndrome patients, and in 4 of 17 samples from 16 Bell's palsy patients. Three of these four positive patients were thought to have zoster sine herpete because of hearing difficulty, vertigo, and pain. VZV IgM antibodies were positive in 1 of the 2 patients with Ramsay Hunt syndrome, and in 2 of the 17 samples from the Bell's palsy patients. VZV IgG antibody titers during the acute phase were significantly higher in the patients positive for the PCR or VZV IgM antibody than in those negative for them. These findings provide evidence that Bell's palsy in some patients could be associated with VZV reactivation. ***J. Med. Virol. 56:359–363, 1998.***

KEY WORDS: varicella-zoster virus; polymerase chain reaction; Ramsay Hunt syndrome; Bell's palsy; facial nerve palsy; zoster sine herpete

INTRODUCTION

Ramsay Hunt syndrome is characterized by facial nerve palsy with auricular vesicles, vertigo, and hearing difficulty due to reactivation of varicella-zoster virus (VZV), whereas Bell's palsy is characterized by facial nerve palsy without auricular lesions and is due to unknown causes. However, based on the observation that significant alterations in VZV antibody titers are occasionally demonstrated in some patients with Bell's palsy, it is thought that Bell's palsy in some patients could be associated with reactivation of VZV, that is, zoster sine herpete. In a few reports, significant changes in specific antibodies or a positive IgM antibody have been observed in 8–25% of patients with Bell's palsy [Tomita et al., 1972, 1988; Djupesland et al., 1976; Kukimoto et al., 1988]. Morgan et al. [1992] reported that 84% of patients with Ramsay Hunt syndrome and 9.3% of patients with Bell's palsy exhibited significant alterations in the antibody. Therefore, it can be assumed that in some cases, Bell's palsy may be related to reactivation of VZV. There have been few reports concerning virological evidence of reactivation of latent VZV except for alterations in VZV-specific antibody titers. This study was undertaken to obtain stronger virological evidence of VZV reactivation in some patients with Bell's palsy.

MATERIALS AND METHODS

The subjects were 16 otherwise normal patients with Bell's palsy and 2 otherwise normal ones with Ramsay Hunt syndrome, who were diagnosed in the Department of Pediatrics or the Department of Otorhinolaryngology of Kawasaki Medical School Hospital between October 1994 and December 1997. One of the patients contracted Bell's palsy twice. Blood samples were drawn within 5 days after the onset of facial nerve palsy and swab samples were obtained at the same time from auricular lesions of Ramsay Hunt syndrome. We excluded those patients who had underlying diseases, which could influence immunity, or who were

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TABLE I. Results of PCR and VZV IgG and IgM Antibody in Patients with Ramsay Hunt Syndrome[a]

		PBMC		Auricular lesions		IgG antibody (IU/mL)		IgM antibody	
Patient	Age, years	Single	Double	Single	Double	Acute	Convalescent	Acute	Convalescent
1	10	–	+	+	+	2100	3500	+	+
2	82	–	+	+	+	4700	nd	–	nd

[a]PCR, polymerase chain reaction; single, single PCR; double, double nested PCR; nd, not done; VZV, varicella-zoster virus; PBMC, peripheral blood mononuclear cell; +, positive; –, negative; IU, international unit.

first seen 6 or more days after the onset of the facial nerve palsy. The control subjects were 20 healthy adults under 50 years of age with a past history of chicken pox and 20 healthy children who had contracted chicken pox at over 1 year of age. Informed consent was obtained from the parents of the children and from the adult patients according to the guidelines of the United States Department of Health and Human Services and those of Kawasaki Medical School.

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood by ficoll-hypaque gradient centrifugation. The swab samples from auricular lesions were obtained by scratching the lesions with a swab to break the vesicles. Then the swabs were soaked in 2 ml of RPMI 1640 for 1 hr. Next, DNA extraction was carried out from 2×10^6 cells of PBMC and 1 ml of RPMI 1640 using the SepaGene kit (Sankou Junyaku, Tokyo). The PCR was done as described previously [Terada et al., 1995], using oligonucleotide from VZV gene 31, which codes for the viral glycoprotein II. For the nested double PCR, 1 μl of the amplification products in the first PCR using the outer primers was added to the new reaction mixture with inner primers to do the second PCR. The sequences of the inner and outer primers were 5′-TCCAACCAACCGTTAAATGA-3′ and 5′-TTGAAGCCAGATCAATATTC-3′, 5′-GAGGAAGTTGAAGCCAGATC-3′ and 5′-CTTCCAGTTCCAACCAACCG-3′, respectively.

The PCR was carried out under standard reaction conditions with denaturation at 95°C for 1 min, reannealing at 60°C for 30 sec, extension at 72°C for 1 min for 30 cycles, and a final 7-min extension interval. The second PCR was done under the same conditions for 25 cycles. All reactions were carried out in a programmable cyclic reactor Omn-E (Hybaid, Middlesex, UK). The PCR controls included human cellular DNA extracted from the PBMC of healthy children without the VZV IgG antibody, the reaction mixture alone, and a positive control of VZV. PCR amplification products were detected by direct gel analysis with ethidium bromide staining after each PCR. After electrophoresis on a 2.5% agarose gel, a band of 235 base pairs was seen when samples contained VZV DNA. The amplified products of the positive band could be detected by dot-blot hybridization using the probe of 5′-GCCTACGGGATGGTGCATAC-3′ labeled with ^{32}P [Koropchak et al., 1991]. In this system, VZV DNA could be detected at a level of approximately 50 copies. To minimize contamination of the PCR in the experiment, we used PCR-sterile conditions in a separate room and a clean ventilation booth only for the PCR. The serum levels of the VZV-specific IgG and IgM antibodies were measured with an ELISA kit (Enzygnost, Hoechst Japan Pharmaceutical, Tokyo) using the α-method. A twofold increase in the titers in paired sera is thought to be significant. All samples were measured in the same assay, and the second and third paired samples were retested with the first samples.

RESULTS

The results of the PCR in patients with Ramsay Hunt syndrome or Bell's palsy are shown in Table I and II. We detected VZV DNA in the auricular lesions of both patients with Ramsay Hunt syndrome by the single PCR and the double nested PCR, and in PBMCs by the double nested PCR, but could not detect it in PBMCs by the single PCR. In contrast, VZV DNA was detected in PBMCs from 4 of 17 samples from the 16 patients with Bell's palsy by the double nested PCR. VZV DNA was not detected in the control subjects.

The VZV-specific IgG and IgM antibody results are shown in Tables I and II. The VZV IgM antibody was positive in 1 of the 2 patients with Ramsay Hunt syndrome and in 2 of the 17 samples from the patients with Bell's palsy. There were two cases positive for the VZV IgM antibody among six cases positive for the PCR. On the other hand, among three cases positive for the VZV IgM antibody, there were two cases positive for the PCR. The VZV IgG antibody titers in the acute phase shown in Figure 1 were significantly higher in the patients positive for the PCR or VZV IgM antibody than in those negative for them ($P < 0.002$, Mann-Whiteney U test). Three of the four patients with Bell's palsy who were positive for the PCR (patients 9, 13, and 16) were thought to have zoster sine herpete because patient 9 experienced pain, vertigo, and hearing difficulty, and patients 13 and 16 complained of earache with Bell's palsy.

DISCUSSION

Although the etiology of Bell's palsy remains unknown, viruses of the herpes group, especially VZV and herpes simplex type 1 (HSV-1), are most commonly implicated as infectious causes of Bell's palsy. In Ramsay Hunt syndrome, it is believed that VZV is associated with facial nerve palsy because of isolation of the virus from the auricular vesicles. In addition, administration of acyclovir is effective for the treatment of Ramsay Hunt syndrome [Stafford et al., 1986; Inamura et al., 1988; Murakami et al., 1997]. Reactivation of VZV is thought to occur in 8–25% of patients with Bell's palsy, as is the case in Ramsay Hunt syndrome. This conclu-

TABLE II. Results of PCR and VZV IgG and IgM Antibody in Patients With Bell's Palsy[a]

		PBMC		IgG antibody (IU/mL)		IgM antibody	
Patient	Age, years	Single	Double	Acute	Convalescent	Acute	Convalescent
3	3	–	–	<100	<100	–	–
	4	–	+	860	1600	+	+
4	5	–	–	920	nd	+	nd
5	44	–	–	410	nd	–	nd
6	70	–	–	510	nd	–	nd
7	26	–	–	290	370	–	–
8	14	–	–	240	nd	–	nd
9	56	–	+	1700	nd	–	nd
10	13	–	–	350	240	–	–
11	48	–	–	450	nd	–	nd
12	1	–	–	<100	<100	–	–
13	73	–	+	930	1700	–	–
14	22	–	–	390	370	–	–
15	29	–	–	160	120	–	–
16	23	–	+	820	nd	–	nd
17	4	–	–	<100	<100	–	–
18	24	–	–	460	nd	–	nd

[a]Patients 9, 13, and 16 were thought to have experienced zoster sine herpete because of Bell's palsy with hearing difficulty, vertigo, and pain. A twofold increase in VZV IgG antibody titers is thought to be significant when this method is used.

sion is based on the observation of significant changes in the VZV antibody in some of these patients [Tomita et al., 1972, 1988; Djupesland et al., 1976; Kukimoto et al., 1988]. Furuta et al. [1997] reported that VZV DNA was detected using the PCR in saliva from 6 (17%) of 36 patients with Bell's palsy. Saliva is an easily obtained sample, but contamination is inevitable, since VZV DNA has been detected in air samples from rooms housing patients with chicken pox or zoster, and even outside hospital isolation rooms on some occasions [Sawyer et al., 1994]. Therefore, to obtain stronger virological evidence of VZV reactivation from easily obtained and less contaminated samples for early diagnosis of zoster sine herpete, we tried to detect VZV DNA in swab samples and/or PBMCs from patients with Bell's palsy or Ramsay Hunt syndrome.

VZV is cell-associated in nature. Ozaki et al. [1986] isolated VZV from PBMCs in patients with chicken pox, although it is very difficult to isolate from the blood itself. The positive rate for the double nested PCR for VZV DNA in PBMCs is from 78% to 100% [Ozaki et al., 1994; Terada et al., 1995] in the acute phase of chicken pox. In herpes zoster, that is, reactivation of VZV, we demonstrated VZV DNA in PBMCs from 5 of 11 patients with zoster within 5 days from the onset (data not shown). Gilden et al. [1992] reported that VZV DNA in PBMCs was detected in patients with postherpetic neuralgia years after rash and with the absence of zoster. Wilson et al. [1992] reported detection of VZV DNA in PBMCs from VZV seropositive bone marrow recipients by PCR and obtained virological evidence of subclinical reactivation of the virus, which had been previously suggested by significant changes in antibody titers [Ljungman et al., 1986]. Subclinical reactivation of VZV has been reported in subjects with a high risk of herpes zoster (children with malignancies and healthy children who contracted chicken pox at under 1 year of age) using PCR or the antibodies [Terada et al., 1994, 1995]. Therefore, PCR in PBMCs for VZV DNA is sensitive not only in patients with chicken pox and zoster but also in those with subclinical reactivation and zoster sine herpete.

In this study, we were able to detect VZV DNA in PBMCs from 4 (23%) of 17 samples from patients with Bell's palsy and both patients with Ramsay Hunt syndrome and from the auricular lesions of the latter patients. In the auricular lesions from these patients, VZV DNA was demonstrated by the single PCR, but in the PBMCs from patients with Ramsay Hunt syndrome or Bell's palsy the double nested PCR was necessary to demonstrate it. These findings suggest that there is less VZV DNA in PBMCs than in auricular lesions. VZV DNA was not detected in the control subjects. Three of the four patients with Bell's palsy who were positive for the PCR were clinically thought to have zoster sine herpete because of pain in the ear canal, vertigo, and hearing difficulty with facial nerve palsy, but no one who has negative for the PCR and VZV IgM antibody complained of the above symptoms, except for facial nerve palsy.

A twofold increase in VZV IgG antibody titers is thought to be significant when the α-method is used. Alterations in the VZV IgG antibody titers showed an almost twofold increase in the paired sera of patients 1, 3, and 13, who were positive for the PCR. The VZV IgG antibody was significantly higher in patients positive for the PCR or VZV IgM antibody than in those negative for them. This suggests that they had already been boostered in the acute phase of Ramsay Hunt syndrome or zoster sine herpete. In this study, the VZV IgM antibody was recognized in two of six patients positive for the PCR. Cradock-Watson et al. [1979] reported that 78% of patients with herpes zoster have the VZV IgM antibody. These findings provide evidence that Bell's palsy in some patients could be associated with VZV reactivation. However, the VZV IgM anti-

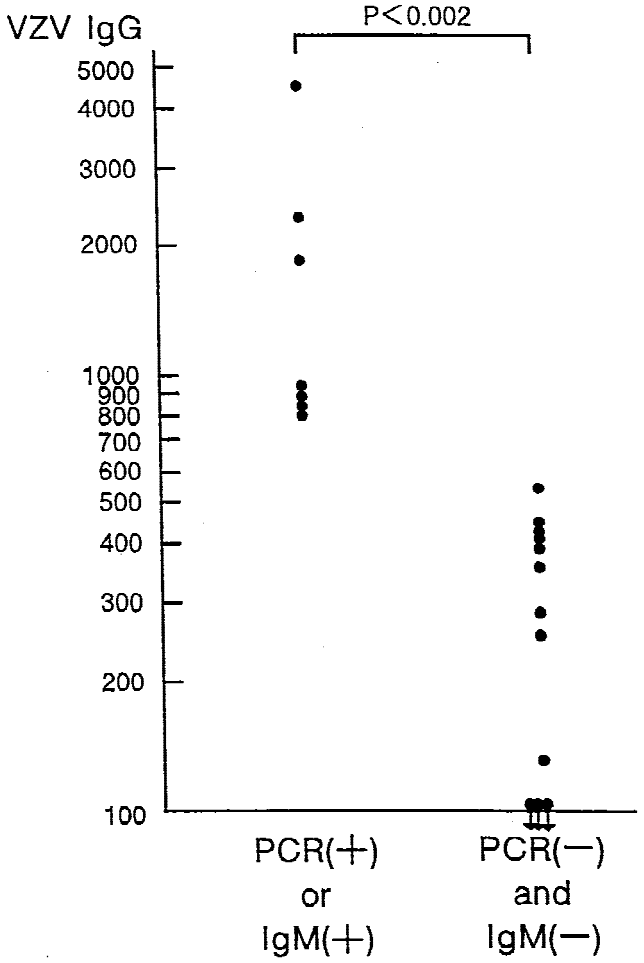

Fig. 1. Varicella-zoster virus (VZV)-specific IgG antibody titers in the acute phase of Ramsay Hunt syndrome or Bell's palsy. PCR(+) or IgM(+), in patients positive for the PCR or VZV IgM antibody; PCR(−) and IgM(−), in patients negative for the PCR and VZV IgM antibody.

body was not detected in all cases with zoster and PCR was not always positive in patients positive for the VZV IgM antibody. The PCR and IgM antibody results together can compensate for each other and increase the frequency of a diagnosis of zoster sine herpete.

Murakami et al. [1996] reported that HSV-1 DNA was detected by PCR in facial nerve endoneurial fluid and posterior auricular muscle from 11 of 14 patients with Bell's palsy. In the usual setting, however, it is extremely difficult to obtain the nerve endoneurial fluid or posterior auricular muscle of patients with Bell's palsy. Furuta et al. [1998] reported that the positive prevalence of HSV-1 DNA in saliva from patients with Bell's palsy (50%) as measured by the PCR was significantly higher than that in healthy volunteers (19%, $P < 0.05$). Therefore, HSV-1 could also be associated with Bell's palsy. In this study, we could not detect HSV-1 DNA in PBMCs from 10 patients with Bell's palsy by the nested PCR, which was done by the same method of Furuta et al. [1998]. However, the HSV-1–specific IgG antibody increased significantly in patient 3 among the nine patients with Bell's palsy whose paired sera were measured. This patient had formerly experienced facial nerve palsy. The IgM antibody was not detected in any cases of Ramsay Hunt syndrome or Bell's palsy. Acyclovir is effective against VZV and HSV-1. If HSV-1–associated facial nerve palsy and zoster sine herpete could be diagnosed at the early stage, it would be possible to treat patients with facial nerve palsy associated with VZV or HSV-1 with antiviral drugs, thus lessening the morbidity and duration of the disease.

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